Alpha4对蛋白磷酸酶2A的调节作用研究进展

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摘要 蛋白磷酸酶2A(protein phosphatase 2A, PP2A)是真核细胞内重要的丝氨酸/苏氨酸蛋白磷酸酶, 调控着许多重要的细胞生命活动。近年来, 随着PP2A逐渐被认为是一个必要的肿瘤抑制因子, alpha4作为其重要的一个调节蛋白也受到了广泛关注。Alpha4能对PP2A进行多种调节, 包括调节PP2A的酶活性和促进PP2A核心酶的组装。在转化细胞和肿瘤细胞中还发现了alpha4的异常表达, 这表明alpha4具有促进肿瘤发生的功能。该文就哺乳动物中alpha4对PP2A的调节机理作一综述, 这将有助于我们更好地理解PP2A和alpha4的作用机制及该机制对肿瘤的治疗意义。

关键词 蛋白磷酸酶2A; alpha4; 调节作用

Progress on Regulation of Protein Phosphatese 2A by Alpha4

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Abstract Protein phosphatase 2A (PP2A), as one of the most important serine/threonine phosphatases in eukaryotic cells, plays crucial roles in the process of cell cycle and signaling transduction. Numerous studies indicate that PP2A acts as a tumor suppressor and, therefore, the PP2A binding protein alpha4 starts being given more attention. Alpha4 has been reported to interact with PP2A, as it maintains the stability of PP2A activity and manages core enzyme assembly of PP2A. Moreover, alpha4 mutants are discovered in carcinogen-transformed human cells and primary human cancer cells, which demonstrate the character of carcinogenesis of alpha4. In this review, a brief summary of advances in studies of regulation of PP2A by alpha4 is given, which might help to give a further comprehension of PP2A and alpha4 in basic and clinical investigations for cancer.

Keywords protein phosphatase 2A; alpha4; regulation function

蛋白磷酸酶2A(protein phosphatase 2A, PP2A)是一个在真核细胞中广泛表达的高度保守蛋白, 占细胞总蛋白含量的0.3%~1.0%[1-2]。作为磷酸化蛋白磷酸酶(phosphoprotein phosphatases, PPPs)家族中的重要一员, PP2A是细胞内主要的丝氨酸/苏氨酸蛋白磷酸酶, 参与调控了许多细胞生命活动, 例如DNA复制、转录和翻译、信号转导、细胞增殖、细胞凋亡、细胞周期以及细胞骨架的动态调控等[1]。PP2A由催化亚基PP2A/C、结构亚基PP2A/A和调节亚基PP2A/B组成, 有二聚体和三聚体两种存在形式。PP2A/C和PP2A/A结合形成的二聚体形式称为AC核心酶, AC核心酶再与PP2A/B结合形成PP2A三聚体全酶。PP2A/C和PP2A/A分别有α、β两种异构体。最近还报道发现了PP2A/Cα的一个基因剪切异构体, 叫做PP2A/Cα2。研究认为, PP2A/Cα2不能与PP2A/A结合, 因此无法组装为PP2A全酶, 但PP2A/Cα2可以与alpha4(α4)结合[3-4]。该剪切异构体表达的条件还未明确, 可能与血清饥饿有关, 当处于恶劣环境下时, 剪切体被触发, 进而对PP2A/Cα的RNA进行修改[4-6]。最近研究发现, PP2A/Cα2与慢性淋巴细胞白血病

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(chronic lymphocytic leukemia, CLL)有较大相关性[6]。PP2A/B有超过26种异构体, 主要分为B(PR55/B)、B'(PR61/B')、B''(PR72/B'')、B'''(PR93/B''')四个亚家族, 并表现出时间和空间上的表达特异性, 其多样性影响着PP2A全酶的亚细胞定位和底物专一性[1,7]。由不同亚基组合形成的PP2A三聚体全酶至少超过96种, 这极大地丰富了PP2A的功能和特异性。近年来, 有研究报道, PP2A负性调节许多促肿瘤信号通路中的重要蛋白质, 例如Akt、c-Myc和Bcl-2等, 同时, PP2A的失调能够诱导细胞的转化[8]。在肿瘤细胞内, 曾观察到PP2A异常表达、遗传突变以及酶活性异常等现象[9], 表达PP2A亚基的某些基因也出现了较高的突变现象, 尤其表达PP2A/A的基因的突变能够干扰AC核心酶和PP2A三聚体全酶的组装, 进而影响PP2A功能的发挥[10]。因此, PP2A又被认为是一种肿瘤抑制因子。

PP2A酶活性受多种方式调控, 除调节亚基多样性和自身亚基的翻译后修饰外, 与某些调节蛋白的结合也能影响PP2A酶活性[1], alpha4就是PP2A的一个非经典型调节亚基(non-canonical regulatory subunit), 它不仅能够调节PP2A的酶活性和全酶组装(图1), 还被认为具有促进肿瘤发生的功能[11], 因此受到广泛关注。本文将围绕目前哺乳动物中alpha4对PP2A的调节作用的研究进展作一综述。

1　Alpha4简介

Alpha4是酵母细胞中Tap42蛋白质的人类同源物, 最初它被认为是哺乳动物B细胞和T细胞淋巴球受体细胞转导复合物中的一个组分, 因此又叫做免疫球蛋白结合蛋白1(immunoglobulin-binding protein 1, IGBP1)。但人们随即发现, alpha4的表达和分布是十分广泛的, 敲除*alpha4*具有胚胎性致死[12-14]。PP2A家族包括PP2A、PP4和PP6三种蛋白磷酸酶, alpha4是PP2A家族共有的一个非特异性调节亚基结合蛋白[15-16]。Alpha4能够调节许多细胞生命活动, 在酵母细胞中, Tap42作为Tor信号通路中的重要靶点调节生长因子和营养物质刺激下的细胞生长和代谢[13]; 而在哺乳动物细胞中, alpha4参与了细胞应激和DNA修复过程, 保护细胞不受一系列的压力刺激, 它还促进PP2A全酶合成, 稳定AC核心酶和PP2A/C[17-18]。alpha4的表达还与胰岛素信号通路途径有关[19]。近几年的研究发现, alpha4具有促进肿瘤发生的作用, alpha4能够抑制细胞凋亡和调节细胞伸展和细胞迁移[12,20]。在许多肿瘤细胞中曾观察到alpha4的显著上调, 并同时伴随着细胞迁移和细胞增殖的升高[11,21]。最近研究还发现, alpha4的表达能够增强细胞增殖、迁移和入侵, 进而促进肝癌的发展[22]。

2　Alpha4可以调节PP2A酶活性

Alpha4对PP2A酶活性的调节是非常复杂的。一些研究认为, alpha4能够激活PP2A[23-26]; 另外一些研究则发现, alpha4抑制PP2A[15,17,27-29]。Sents等[30]认为, 这可能与这些研究中采用的PP2A底物不同有关, alpha4实际上通过引起PP2A/C构象的变化改变

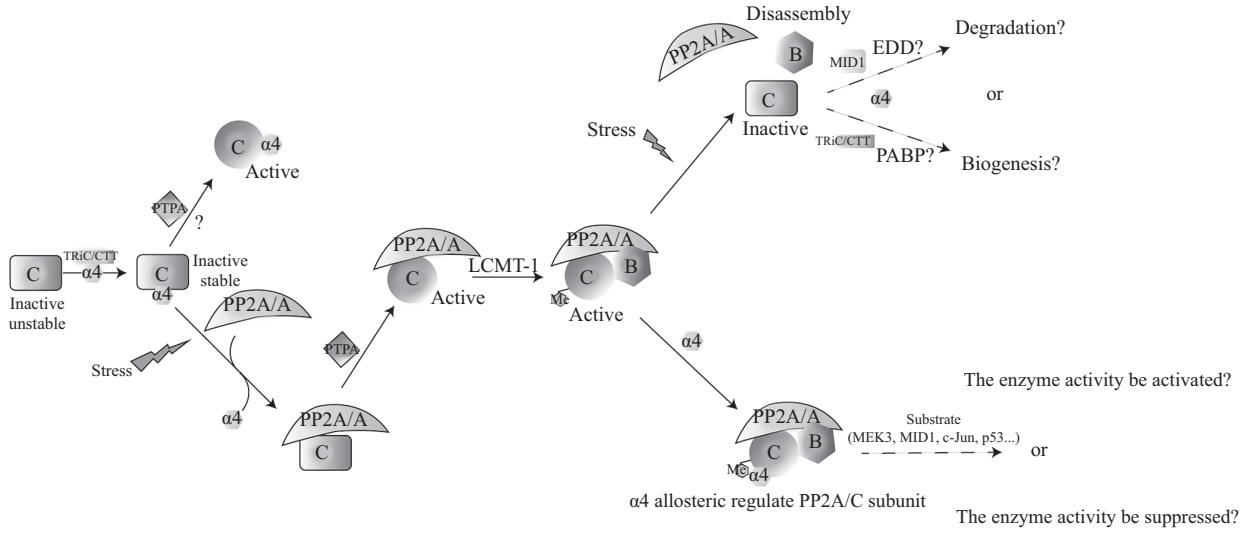

图1　哺乳动物细胞中alpha4(α4)对PP2A的调节作用(根据参考文献[30]修改)

Fig.1　Regulation of protein phosphatese 2A by alpha4 (α4) in mammalian cells (modified from reference [30])

PP2A对底物的特异性。PP2A/C的某些结合抑制剂, 例如冈田软海绵酸和微囊藻毒素, 被报道能够引起PP2A/C和alpha4的解离[31-32]。但也有研究发现, 它们对PP2A/C和alpha4的结合没有明显的影响[33-34]。这提示, alpha4更可能是变构调节PP2A/C, 而非简单地空间封闭其活性位点[30,35]。机体内alpha4的短期上调能够不同程度地升高或降低不同PP2A底物, 如cAMP应答元件结合蛋白(cAMP response element binding protein, CREB)、双链RNA依赖的蛋白质激酶(double-stranded RNA-dependent protein kinase, PKR)、丝裂原活化蛋白激酶(p38 mitogen-activated protein kinase, p38)等的磷酸化水平, alpha4还对PP2A家族的PP2A和PP6进行不同的变构调节[35]。该研究结果显示, 无论底物是pNPP还是MBP, 单体PP2A和单体PP6的催化能力均表现出相同的动力学性质; 然而, alpha4与PP2A的二聚体(alpha4·PP2A)和alpha4与PP6的二聚体(alpha4·PP6)却明显不同: 当底物为pNPP时, 两者均没有任何催化活性, 而当底物为MBP时, alpha4·PP2A展现出了比alpha4·PP6高100倍的V_{max}[35]。以上结果说明, alpha4是PP2A/C的一个变构调节因子。PP2A/A也能变构调节PP2A/C[34], PP2A/A上某些位点的磷酸化还促进其与PP2A/C解离, 进而导致PP2A酶活性降低[36]。但与PP2A/A不同的是, alpha4不需要通过PP2A/B介导它与底物的结合, alpha4自身灵活可变的C-端可以直接结合PP2A底物, 其N-端则与PP2A/C结合, 这使alpha4能够介导调控PP2A对底物的去磷酸化[37-38]。除了与PP2A/C直接结合调节PP2A酶活性外, 研究还发现, alpha4能通过提高调节亚基为B55a的这一PP2A亚型的活性来促进细胞存活, 而包含了所有不同亚型的PP2A整体活性不变, 这提示, 调节亚基B的种类也能影响alpha4对PP2A酶活性的调节效果[32,39]。

3 Alpha4可以与游离PP2A/C亚基结合

与alpha4结合的游离PP2A/C(新合成或由于热激等不稳定因素从全酶复合体上脱离下来的PP2A/C)是少数的, 酵母细胞中约有2%的PP2A/C与约10%的Tap42结合[13]。晶体结构分析发现, 与alpha4结合的游离PP2A/C处于部分未折叠的状态, 且没有催化活性[18]。Kong等[17]利用免疫共沉淀的实验方法发现, 在选用三种不同PP2A底物的实验条件下, 与alpha4结合的游离PP2A/C均是没有活性的, 而与PP2A/A结合的PP2A/C都具有催化活性。Jiang等[18]进一步发现, 正常游离PP2A/C上没有alpha4的结合位点, 因为PP2A/C活性位点附近存在着环状结构和α-螺旋等, 它们在空间上阻碍了alpha4与PP2A/C的结合, 通过移除金属催化离子和空间暴露PP2A/C的N-端区域(nPP2A/C, 氨基酸残基位点1-153)后, 能够形成alpha4的结合位点, 去除这些结构后, 该区域的未折叠状态使alpha4能够与nPP2A/C结合, 但这同时也改变了PP2A/C活性位点的正常构象, 导致其失去催化活性。Alpha4与游离PP2A/C结合并使其稳定在无活性构象, 这确保了在形成具有底物特异性的PP2A全酶之前, 游离PP2A/C的活性能够得到控制。Alpha4上与游离PP2A/C结合的区域是一个全α-螺旋形区段, 它在结构上与14-3-3蛋白和三角形四肽重复蛋白(tetratricopeptide repeat-like protein, TPR)十分相似, 且该区段上带正电荷的碱性氨基酸残基(Arg155、Arg163、Lys158、Lys163、Lys166)对alpha4与PP2A/C的结合至关重要, 它们能与游离PP2A/C上带负电荷的酸性氨基酸残基结合[34,37,40-42]。Sents等[30]猜测, alpha4与PP2A/C的这一无活性复合体结构能够被PP2A激活物(PP2A phosphatase activator, PTPA)激活, 且具备催化底物蛋白质去磷酸化的活性。

Alpha4与游离PP2A/C的结合竞争性取代PP2A/A和PP2A/B[13,24,34,37]。Nanahoshi等[15]研究曾发现, PP2A/C能够与alpha4或与PP2A/A结合, 但三者并不形成三聚体复合物。在酵母细胞内, PP2A/A的下调能够引起Tap42与PP2A/C结合的增加[13,43-44]; 反之, alpha4与PP2A/C的解离能够引起PP2A/A与PP2A/C结合增加[34]。之前的研究认为, alpha4和PP2A/A之所以不能同时与PP2A/C结合, 是因为PP2A/C上alpha4的结合位点与PP2A/A的结合位点部分重叠[34]。进一步研究后, Jiang等[18]有了更为详细的解释, 他们发现, alpha4在与游离PP2A/C结合后, PP2A/C通过中间区域β-折叠的变构延迟(allosteric relay), 改变了相反面上PP2A/A结合位点的构象, 从而导致PP2A/A无法结合。PP2A/C的这一特性确保了其对alpha4和PP2A/A的专一性结合, 这使alpha4对游离PP2A/C的调节更为准确。

4 Alpha4可调节PP2A/C亚基的泛素化降解

细胞内的PP2A/C在转录翻译水平上受到机体严密的自我调控, 外源性过表达或敲低不能改变

其含量, 但单体的PP2A/C却极易被泛素化降解[45]。Trockenbacher等[46]早前曾报道, 具有E3泛素连接酶活性的MID1能够通过alpha4的“支架”作用与PP2A/C相互联系, 并因此介导PP2A/C的泛素化降解。Watkins等[47]还发现, alpha4催化PP2A/C的蛋白酶体降解。这些表明, alpha4能够介导催化PP2A/C的降解。然而, alpha4同时也能保护PP2A/C不受泛素化降解。Kong等[17]曾观察到, alpha4的下调能够引起细胞内PP2A、PP4、PP6、PP2A/C、PP2A/A含量显著下降, PP2A酶活性也随之降低。Eleftheriadou等[48]在H9c2心肌细胞中将alpha4短期敲低后观察到了PP2A、PP4和PP6含量的显著下降。在神经鞘氨醇磷酸胆碱(sphingosylphosphorylcholine, SPC)诱导的角蛋白8的过度磷酸化和重构研究中, Lee等[49]发现, SPC能够降低PP2A与a4的结合, 进而促进PP2A的泛素化降解, 这一过程与SPC诱导的小窝蛋白-1(caveolin-1, cav-1)的表达升高有关。Park等[50]最近也报道了蛙皮素(cerulein)能够通过下调alpha4的表达水平和上调PP2A的泛素化来使细胞内PP2A表达水平下降。综上, alpha4能够调节PP2A/C的泛素化降解, 这一调节主要是alpha4通过调控MID1和EDD对PP2A/C的泛素化降解实现的。

4.1 Alpha4调控MID1对PP2A/C亚基的泛素化降解

MID1是拥有E3泛素连接酶活性的一个微管相关蛋白(microtubule-associated protein, MAP), 它与奥皮茨综合征(Opitz syndrome)的发生有着紧密关联。MID1上N-端区域的RING、Bbox1、Bbox2三个锌结合区域(zinc-binding domains)提供了MID1的E3泛素连接酶活性[51]。MID1的Bbox1区域与alpha4的C-端区域中一段包含45个氨基酸残基的区段(alpha45)紧密结合, 而alpha4的N-端区域结合着PP2A[52]。MID1的Bbox1区域上P151L(the XLOS-observed proline 151 to leucine)的突变与奥皮茨综合征的发生有关, 该位点突变后不改变Bbox1区域的空间结构, MID1的E3泛素连接酶活性仍然得到保留, 但MID1失去与alpha4结合的能力[53]。MID1与alpha4解离后的效应是多样的, 除了具有促进奥皮茨综合征发生等恶性效应外, 两者的解离也能对机体的其他病变产生拮抗作用。研究曾发现, MID1-alpha4-PP2A/C复合物受到破坏后, 对神经性疾病(例如阿尔茨海默症)具有缓解和治疗的效果[54]。前列腺癌细胞中, 二甲双胍诱导的MID1与alpha4结合的破坏还被发现具有抑制肿瘤的作用[55]。通过中间的alpha4“支架”, MID1一方面催化PP2A的泛素化降解, 另一方面它受PP2A作用被脱磷酸化, 以此实现其细胞功能的正常行使[46,51,56]。在细胞内, 少量的MID1突变会造成其与alpha4-PP2A/C复合物分离, 但不会破坏MID1与微管的结合, 这引起MID1的高磷酸化水平及其在微管上转运功能的丧失[57]。大量的MID1突变则会破坏MID1与微管的结合, 但MID1仍与alpha4-PP2A/C复合物结合, 因此导致许多MAP大范围的去磷酸化以及微管相关的PP2A/C含量的显著升高[46,58]。

文献曾报道, alpha4能够促进MID1对PP2A/C的泛素化降解[46-47]。但后来一些研究发现, alpha4实际上抑制了MID1对PP2A/C的泛素化降解[17,56]。关于这一问题, McConnell等[56]作出了解释。他们发现, 在野生型alpha4的氨基酸序列46~60位点区段存在着一个泛素分子结合结构域(ubiquitin-interacting motif, UIM), UIM虽然与单泛素化的PP2A/C结合, 但它阻止了泛素分子进一步与PP2A/C结合, 因此抑制了PP2A/C的多泛素降解[30,40,56]; 当丢失UIM后, alpha4反而与多泛素化蛋白(polyubiquitinated protein)的结合增加, 失去了对PP2A/C的保护作用[56]。含UIM的alpha4也与一个泛素分子结合, 呈现单泛素化状态[56], 这一单泛素化位点可能位于其C-端的一个Lys氨基酸残基上, 该位点附近的一段45氨基酸残基多肽被单泛素化后, 能够抑制MID1的催化活性, 降低多泛素化产物的数量[51]。Sents等[30]推测, 该多肽是通过抑制MID1与E2泛素结合酶的结合实现对MID1活性抑制的。除UIM外, 在alpha4对PP2A/C的泛素化保护作用中, alpha4上N-端PP2A/C结合区域和C-端MID1结合区域的完整性也是不可缺少的[40]。

4.2 Alpha4调控EDD对PP2A/C亚基的泛素化降解

EDD(E3 isolated by differential display)基因是E3泛素连接酶基因的家族成员之一, 最初它被认为是人类T47D乳腺癌细胞中的一个受孕酮诱导的基因(progestin-induced gene), 在乳腺癌细胞中, 孕酮等激素能够上调EDD的表达[59-61]。McDonald等[62]利用酵母双杂交实验发现, EDD可以与alpha4结合。通过进一步研究, McDonald等[59]发现, alpha4的N-端区域结合着PP2A/C, 而PP2A/C的两个E3泛素连接酶(EDD和MID1)均与alpha4的C-端区域结合。细胞内的alpha4是被单泛素化的[56,59], MID1能够催化alpha4的单泛素化[51]。Alpha4虽然与EDD存在物理

结合, 但单泛素化的alpha4并不是EDD的底物; EDD不与PP2A/C直接结合, 但EDD却能催化PP2A/C的多泛素化[59]。关于EDD催化PP2A/C多泛素化降解的机制尚不明确, McDonald等[59]推测, 这与EDD含有的RING和HECT结构域有关, 认为即使在EDD和PP2A/C没有直接结合的情况下, 利用这两个特殊的结构域, 再加上alpha4的介导作用, EDD也能将PP2A/C泛素化降解。除了alpha4介导的EDD对PP2A/C的泛素化降解调节作用外, 由于孕酮等激素能够诱导EDD的表达, 细胞内可能还存在通过激素对PP2A/C含量的间接调控方式[59]。

4.3 Alpha4调节PP2A/C亚基泛素化降解的其他可能机制

除了上述相关机制, 一些研究还检测到, alpha4蛋白与PP2A/C的结合覆盖了PP2A/C上作为多泛素化位点的赖氨酸残基(Lys41), 认为这也保护了PP2A/C不被多泛素化降解[18]。此外, 实验发现, 缺失MID1能够导致细胞内PP2A/C含量增加[63], 所以可能存在alpha4直接通过调节MID1的E3泛素连接酶活性来保护PP2A/C的调节方式[30,51]。尽管如此, 可能只有少量的alpha4对于维持PP2A的含量水平是必需的[16]。Sents等[30]认为, alpha4对于PP2A/C并不是绝对的保护作用。因为在某些正常情况下, alpha4反而促进PP2A/C的降解[46]。alpha4上N-端区域存在的UIM虽然可以保护PP2A/C不被泛素化, 但alpha4的C-端招募结合的EDD和MID1却能够促进PP2A/C的泛素化降解[59]。因此, alpha4更可能是细胞内PP2A/C含量的一个“调制器”, 它根据机体的需求, 控制着细胞内PP2A/C的水平, 使其维持在某一稳定水平, 机体对alpha4的修饰(例如翻译后修饰等)可能最终决定了alpha4不同的调节作用, 这些亟待进一步探究。此外, MID1能够通过调节alpha4的单泛素化来改变alpha4的功能。例如, MID1通过将alpha4单泛素化能够催化钙蛋白酶介导的对alpha4的断裂损伤, 这使得alpha4对PP2A的调节功能从保护变为破坏, 进而引起许多MAPs, 尤其是Tau蛋白的高磷酸化, 最终可能导致某些相关神经变性疾病的发生[47]。MID1也能直接催化alpha4的多泛素化降解, 从而负性调节PP2A[47,64]。MID1上的E3结合域(E3 domains)能够催化alpha4的单泛素化, 全长的MID1则能够催化alpha4的多泛素化降解, Bbox1结构域对于MID1催化alpha4的多泛素化降解是必需的, 但该结构域的突变不影响MID1对PP2A/C的多泛素化降解[64-65]。

5 Alpha4可调节AC核心酶的组装和稳定

除了对PP2A酶活性和对PP2A/C稳定性的调节外, alpha4还能促进AC核心酶的组装和稳定[7,17], 并与其他机制联合, 调节PP2A全酶的组装。Kong等[17]曾报道, 正常情况下, alpha4蛋白能够与PP2A/C结合(新合成的或由于热激等不稳定因素从AC核心酶上脱离下来的PP2A/C), alpha4的这一结合不仅抑制了PP2A/C的催化活性, 还保护其不被蛋白酶体降解; 当细胞受到外界压力刺激时, 细胞内的PP2A全酶变得不稳定, 其酶活性受到影响而下降, 此时alpha4迅速将PP2A/C释放, 促进AC核心酶的装配并将其稳定。在此之后, 细胞通过其他调节机制, 例如对PP2A/C的翻译后修饰(磷酸化和甲基化等), 招募相应的PP2A/B与AC核心酶结合, 最终形成PP2A三聚体全酶, 弥补下降的酶活性, 使生命活动正常进行。最近, Wong等[66]在使细胞饥饿状态后, 不仅检测到了alpha4与PP2A/C的解离, 同时还观察到调节亚基为B55a的这一PP2A亚型活性的升高。尽管如此, alpha4与PP2A/C的解离只能在一定程度内调节性上调PP2A酶活性, 解离程度如果进一步加深, 反而可能导致AC核心酶的不稳定, 引起PP2A酶活性下降[67]。

Alpha4与PP2A/C一定程度的解离能够促进AC核心酶的组装和稳定, 这是促进PP2A全酶组装的第一步。PP2A/C上C-端的TPDYFL(304-309)区域是一段高度保守的序列, 对它的翻译后修饰被报道能够影响PP2A/B的结合[68], 虽然目前尚有许多不明确的内容, 但其中已经比较明确的是Thr304位点和Tyr307位点的磷酸化以及Leu309位点的甲基化。Thr304位点和Tyr307位点的磷酸化一般被认为能够阻碍某些亚型PP2A/B与AC核心酶的结合, 从而抑制PP2A酶活性, 例如, Thr304位点的磷酸化可能选择性抑制了PR55/B亚家族与AC核心酶的结合, 而Tyr307位点的磷酸化可能影响着PR61/B’与AC核心酶的结合[68]。Stanevich等[69]报道过PP2A/C的甲基化对PP2A全酶组装的作用, 他们发现, 金属离子与PP2A/C活性位点的螯合以及PTPA与PP2A/C的活性位点的结合, 能够使PP2A/C的活性位点恢复正常的活性构象, 此时PP2A/C被激活。亮氨酸羧基甲基转移酶-1(leucine carboxyl methyltransferase-1, LCMT-1)能够识别这一活性构象并直接结合, 进而

催化PP2A/C上Leu309位点的甲基化, 并招募相应的一些PP2A/B与之结合, 使其最终转变成具有底物特异性的PP2A全酶。细胞中一部分参与了PP2A全酶组装的成熟PP2A/C被报道是处于甲基化状态的[70-71], 且alpha4的下调能够导致这部分处于甲基化状态的PP2A/C含量下降, 并最终导致PP2A含量下降[17], 同时, PP2A的甲基化受阻也会引起alpha4与PP2A/C的结合增加[25]。因此, 笔者认为, alpha4能够促进AC核心酶的组装和稳定, PP2A/C的甲基化则促进PP2A/B与AC核心酶结合形成PP2A全酶, 这两个调节机制是相辅相成的。与alpha4结合的PP2A/C受到抑制和保护, 而当alpha4将PP2A/C释放后, PTPA等将其恢复催化活性, LCMT-1再识别这一活性构象并与之结合, 最后, LCMT-1与alpha4合力促进PP2A全酶的组装。

6　Alpha4可调节PP2A全酶的底物特异性

在alpha4-PP2A复合体中, alpha4连接着PP2A和PP2A底物蛋白, 这与PP2A三聚体全酶中PP2A/A连接着PP2A/C和PP2A/B十分相似, alpha4和PP2A/A都具有“支架”的作用。Alpha4的N-端区域通过α-螺旋二级结构连接着PP2A, 它的C-端区域则连接PP2A底物, 且alpha4的C-端区域的二级结构不是稳定的, 而是一个具备灵活性和延展性的构象, 这保证了alpha4能够利用其灵活多变的C-端区域识别并结合不同的PP2A底物, 从而介导N-端的PP2A对其去磷酸化[37,52]。X射线晶体结构分析发现, 相比于Tap42, alpha4的N-端区域的结构更为开放和灵活, 并且Tap42上不存在UIM[40]。PP2A/A虽然和alpha4一样具有“支架”的作用, 但它不能像alpha4一样直接特异识别底物并介导对底物的催化, 而是需要通过PP2A/B来实现这一功能[72]。

Alpha4能够识别许多PP2A底物, 比如MID1、MEK3、c-Jun和P53等。在MID1-alpha4-PP2A复合体中, PP2A能够催化MID1的脱磷酸化, 维持其微管相关功能的正常行使, MID1这一功能的失常可能会引起奥皮茨综合征[46,51,58]。Alpha4蛋白能结合MEK3, 介导PP2A对MEK3的脱磷酸化, MEK3的脱磷酸化能够抑制P38 MAPK通路的激活, 最终抑制细胞凋亡[73]。相同地, alpha4能够结合并介导PP2A对c-Jun和P53等转录因子的脱磷酸化, 实现对细胞凋亡的抑制[12]。另一方面, 机体同样可以通过抑制alpha4-PP2A复合物来实现对其他生命活动的调节。例如, 乳铁蛋白(lactoferrin, Lf)在哺乳动物内是一个金属离子结合蛋白, 通常通过诱导细胞凋亡来抑制肿瘤的发生。在肺腺癌细胞系中, 有研究发现, Lf能够与alpha4蛋白结合, 通过破坏alpha4-PP2A复合体, 实现对PP2A酶活性的抑制, 最终引发细胞凋亡[74]。

7　Alpha4可能间接调节PP2A/C的翻译和折叠

许多研究已经发现, 将细胞内alpha4敲低后引起PP2A/C、PP4/C和PP6/C含量下降的程度远远低于将细胞内alpha4敲除, 这提示了alpha4可能是一个特异性的“蛋白质折叠酶”, 它能够直接或间接促进PP2A/C、PP4/C、PP6/C适当的折叠, 因此, alpha4的水平直接影响着这部分PP2A/C、PP4/C和PP6/C的水平, 但不影响与alpha4结合的PP2A、PP4、PP6的水平[16]。多聚腺苷酸尾结合蛋白[poly(A)-binding protein, PABP]是真核细胞内主要的一个mRNA结合蛋白, 它能够与mRNA的多聚腺苷酸尾poly(A), 真核翻译起始因子4G(eukaryotic translation initiation factor 4G, eIF4G)和真核释放因子3a(eukaryotic release factor 3a, eRF3a)结合, 促进mRNA的稳定性和翻译的起始[75-76]。PABP还能够在翻译终止过程中促进释放因子的招募, 并且阻止核糖体对密码子的继续识别[77]。EDD和PABP上均存在着多聚腺苷酸尾结合蛋白的C-端结构域[the C-terminal domain of poly(A)-binding protein, PABC], PABC能够招募含PABP相互作用基序2(PABP-interacting motif 2, PAM2)的某些翻译辅助因子与mRNA的poly(A)尾结合, 例如PABP结合蛋白1(PABP-interacting protein 1, Paip1)和Paip2[77]。酵母双杂交实验发现, EDD和PABP都能与alpha4结合, 且alpha4的N-端区域结合着PABP, 而EDD与alpha4的C-端区域结合[62]。Sents等[30]认为, alpha4通过与PABP的结合可能调控了PP2A/C的翻译过程。此外, EDD在翻译起始过程中能够催化Paip2的泛素化降解, 而Paip2是一个PABP活性的负性调控因子[78], 因此, EDD可以通过调节Paip2来调控PABP的活性。Alpha4可能还通过某些结合蛋白调控PP2A/C的正确折叠。曾有研究鉴定出alpha4-PP2A/C复合物的一个伴侣蛋白——能够与alpha4结合的ATP依赖性分子伴侣复合体TRiC/CTT(T-complex polypeptide-1 ring complex/chaperonin-containing T-complex polypeptide complex)[79], 它能够

与PP2A的调节亚基结合[42]。Sents等[30]推测, TRiC/CTT能够促进PP2A/C和alpha4的正确折叠。

8 结语

PP2A作为细胞内普遍存在的一个丝氨酸/苏氨酸蛋白磷酸酶, 它调控着许多细胞内重要的生命活动, 并且参与多种信号通路的转导。Alpha4在生命活动中的许多功能都与PP2A相关, 同时, PP2A功能的实现也离开不了alpha4对其活性的调节和对其底物的介导。近年来, PP2A成为一个被广泛认可的抑癌因子, 它能够负性调节促肿瘤信号通路, 而且还在肿瘤细胞中出现了遗传突变和功能缺失的现象[5-6]。Alpha4也被认为具有促进肿瘤发生的作用, 在原发性肝癌细胞(87.5%)、原发性肺癌细胞(84.0%)和原发性乳腺癌细胞(81.8%)中均观察到了alpha4的高表达, 免疫缺陷小鼠体内异位表达alpha4后, 研究者们还观察到了肿瘤的形成[17]。综上, 对alpha4和PP2A之间调节机理和作用方式的深入研究是探究PP2A抑制肿瘤机制的重要基础, 这为未来在肿瘤治疗中发现更多的PP2A激活药或肿瘤抑制靶点等提供了可能。

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